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UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION
M. F. MILLER, *Director*

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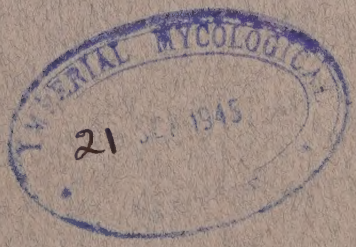
E. R. GARRISON



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CONTENTS

Introduction ..... Page 3

Literature Review 3

Plan of Investigation 5

Results 7

O. lactis in barn air, milk equipment and materials from dairy farms 7

Plate count of O. lactis in fresh cow feces and silage 9

Discussion of results 11

Summary 13

References Cited 14

Sources of *Oospora lactis* on Dairy Farms

E. R. GARRISON

The organism known as *Oospora lactis* is a common contaminant of milk and cream on dairy farms and causes marked deterioration in the quality of cream when extensive growth occurs in this product during holding. The amount of contamination with this mold is influenced by the sanitary conditions under which milk and cream are produced and handled but the important sources of *Oospora lactis* on dairy farms are not well known. A better understanding of these sources should be beneficial in reducing or preventing contamination of milk and cream with this organism during production and handling on the farm.

LITERATURE REVIEW

In 1851 Fresenius[10]* isolated an organism from milk which he named *Oidium lactis* because of its common occurrence in milk and cream. This organism was later placed in the genus *Oospora* by Saccardo[27] and is now usually referred to by the name *Oospora lactis.*

Several early investigators, especially Haberlandt[14], Müller[23], Laser[21], Lang and Freudenreich[20], Arthaud-Berthet[3], Thom[31], Henneberg[16], Gruber[13], Schnell[29] and Düggeli[9] reported that *O. lactis* was usually found in the fresh milk and cream produced on dairy farms and that the numbers increased during aging of these products.

According to Morgan[22], *O. lactis* is perhaps the most common mold associated with dairying. He stated that spores and hyphae fractions of this mold are found in cream and often in samples of milk shortly after it has left the cow. In a study of the fungi found in milk Cummins, Kennelly and Grimes[7] reported in 1929 that *O. lactis, Astrostalagmus cinnabarinus* and a *Phoma* species were the three molds most frequently isolated. In a later report Grimes, Cummins and Kennelly[12] stated that the principal molds found in milk in order of their frequency were *Penicillium, O. lactis, Phoma, Aspergillus* and *Cladosporium* (*Hormodenrum*).

Haberlandt[14], (cited by Schnell[29]) demonstrated in 1875 that *O. lactis* was always present in large numbers in bovine excrement and he therefore, supposed that it existed in significant numbers

*For list of references cited by superscript numerals in text see pages 13 and 14.

in the alimentary tract. Müller[23] found *O. lactis* in the 5 samples of milk examined but was unable to determine if the organism existed in the udder of the cows or entered the milk from the air. The air was regarded by Henneberg[16] as an important source of *O. lactis* infection and Orla-Jensen[24] also reported that this organism was always plentiful in the air of the dairy.

Hansen[15] (cited by Schnell[29]) stated in 1879 that he was able to isolate *O. lactis* from Danish garden soil during most seasons of the year but could obtain it from the air usually only during July and August. In a study of the soil fungi in Germany, Adametz[2] (cited by Schnell[29]) isolated *O. lactis* from the surface layer of the two soils examined. He believed that this mold could easily pass from the soil into milk by feeding potatoes to the cows. *O. lactis* was recovered from 3 out of 7 New Jersey soils studied by Waksman[32, 33] but it was not found in 25 samples from other states. Abbott[1], Dale[8] and Jensen[18] also failed to isolate *O. lactis* from the 40 soil samples investigated.

Arthaud-Berthet[3] reported that *O. lactis* lives on most organic materials in the soil and is present on the fodder and bedding of dairy cows. Bisby, Jamieson and Timonin[4] expressed the opinion that *O. lactis* and other fungi found in butter probably originate in the soil, on plants, in debris and in manure. They believed that cream, butter and dairy equipment were contaminated by spores or bits of mycelium carried in the air and on dust particles.

By means of an enrichment procedure, Schnell[29] regularly obtained *O. lactis* from sewage treated soil, manured soil and garden soil. This mold was also secured on several occasions from fermenting hay, silage, stored grains (barley, corn, oats, rice and rye), potatoes, beets, cabbage, cucumbers and beans. He concluded that *O. lactis* probably occurs on all agricultural products because of its prevalence in the soil and enters milk primarily from the soil, manure and feed. A similar conclusion was reached by Ritter[25] who likewise used an enrichment procedure and demonstrated that *O. lactis* was widely distributed in nature. Isolations of this organism were made by this investigator from the soil, cow manure, garden vegetable plants, compressed yeast and pickle vats.

Geffers[11] made several isolations of *O. lactis* from dairy waste water. According to Robertson[26], *O. lactis* was the principal mold found in the accumulations of old milk in the rubber tubes, stanchion hose and moisture traps of milking machines.

O. lactis has been obtained from a number of miscellaneous sources by the following investigators: Beer, Hansen[15]; sauerkraut brine, Wehmer[34]; decaying vegetables and fruits, Thom[31]; injured areas on lemons, Smith[30]; pickles, sauerkraut and compressed yeast, Henneberg[16]; brewery malt, Bobilioff-Preisser[6] and Hummer[17]; distillery mash, Sito and Naganischii[28]; and overripened tomatoes, Bitting[5].

PLAN OF INVESTIGATION AND PROCEDURE

Barn Air. The relative number of spores, oidia and hyphae fragments of *O. lactis* in the barn air when the dust concentration varied was investigated by the exposure of petri dishes. Ten plates were exposed near the cows or stanchions in each of three representative dairy barns on two different occasions for 10 minutes, (a) while the cows were being brushed and cleaned preparatory to milking, (b) during the milking period and (c) while feeding hay or placing bedding on the stall or stanchion floor. The plates were poured with tomato agar (pH 3.5), incubated 48 hours at 25° C. and examined for colonies of *O. lactis.*

Milk Equipment. The presence of O. lactis in milk equipment during the summer months was studied by visiting cream producing farms near Columbia and wiping a portion of the inner surfaces of the washed, stored equipment with sterile, moistened swabs then replacing the used swabs in the original container and returning them to the laboratory for analysis. The swabs were disintegrated and shaken in the remainder of the 15 ml. of water originally placed in the container, then the cotton and water were distributed among 3 petri dishes. The plates were poured with tomato agar and after incubation observed for *O. lactis.*

The milk tubes and teat cup assembly of milking machines, given the usual cleaning and sterilizing treatments practiced on the individual farms concerned, were examined for *O. lactis* by flushing with 400 ml. of sterile milk, returning the milk to the original flask and inoculating with *S. lactis,* then holding the flasks at room temperature for several days to detect any growth of *O. lactis* on the milk surface.

Feeds, Miscellaneous Materials, Soil and Water. Samples of various feeds, bedding, dirt from barn floors, fresh cow and horse feces, hair and dirt from coat of cows, straw, barnyard

soil, field and garden soils and cistern water were collected in sterile containers at dairy farms in Missouri at different seasons of the year and examined for *O. lactis* by the following method: One pint of whole milk in a quart bottle covered with a bottle cap was steamed in the autoclave for 30 minutes then cooled to room temperature and inoculated with *S. lactis.* When the milk had thickened slightly 10 grams of each material (except dirt from coat of cows in which case 1 gram was used) were stirred into individual bottles and after 3 or 4 days incubation at room temperature the surface growth in each container was examined macroscopically and microscopically for *O. lactis.* Negative samples were given a second inoculation and examination.

Several of the milk cultures of different materials that did not show any surface growth of *O. lactis* were plated in appropriate dilutions on potato dextrose agar but the results were also negative. Plating various dilutions of the original materials on potato dextrose agar was generally unsatisfactory for the detection of this organism because the preponderate number of other molds in most substances usually overgrew and obscured any *O. lactis* colonies on the plates.

During the later part of this investigation it was discovered that tomato agar[19] was an excellent medium for the growth of *O. lactis.* This organism grows so rapidly on plates poured with this medium adjusted to p H 3.5 that the colonies can be recognized and counted after 48 hours incubation at 25° C. before they are obscured by the growth of other molds that usually predominate in feed, soil, manure, etc. This medium was used successfully for making plate count determinations of *O. lactis* in cow feces and silage to ascertain the importance of these materials as a source of this mold on dairy farms.

RESULTS

O. lactis in Barn Air, Milk Equipment and Materials from Dairy Farms

A summary of the results of examinations for *O. lactis* of barn air, milk equipment, feeds, miscellaneous materials, soil and cistern water obtained at dairy farms is shown in Table 1.

Barn Air. *O. lactis* fell from the air into 42 (70.0 per cent) of 60 petri dishes exposed for 10 minutes near the cows in 3 dairy barns while the animals were being brushed and cleaned

TABLE 1.- O. LACTIS IN BARN AIR, MILK EQUIPMENT AND MATERIALS FROM DAIRY BARNS.

Type of sample	No. plates exposed or samples examined	Samples yielding O. lactis	
		No.	Per cent
Barn air when:			
feeding hay or stirring bedding	60	9	15.0
grooming cows	60	42	70.0
milking	60	2	3.3
Equipment			
milk separator	36	29	80.6
milking machines (rubber parts)	39	2	5.1
pails (hand)	60	46	76.7
strainers	23	15	65.2
Feed			
beet pulp	25	2	8.0
concentrates			
barley	24	2	8.3
corn	41	8	19.5
cotton seed meal	24	5	20.8
oats	33	5	15.2
soybean meal	31	3	9.7
wheat and bran	35	9	25.7
summary	188	32	17.0
mixture prepared on farms	50	25	50.0
corn leaves (lower)	30	5	16.7
hay			
alfalfa	36	4	11.1
lespedeza	40	15	37.5
red clover	20	2	10.0
soy bean	23	5	21.7
timothy	22	2	9.1
summary	141	28	19.9
silage	77	51	66.2
Miscellaneous materials			
bedding	36	29	80.6
dirt from barn floors	40	31	77.5
feces			
cow	180	155	86.1
horse	36	30	83.3
hair and dirt from coat of cows*	60	51	85.0
straw	40	8	20.0
Soil			
barnyard	65	51	78.5
field	52	15	28.8
garden	28	13	46.4
Water (cistern)	84	25	29.8

*One gram used for inoculation.

preparatory to milking. The same number of plate exposures made in these barns for 10 minutes when the air was dusty from feeding hay or bedding down the stanchion floor yielded 9 (15.0 per cent) plates that contained this organism. Similar plate exposures made near the cows in the same barns while the animals were being milked gave 2 (3.3 per cent) plates that subsequently developed colonies of *O. lactis*.

Milk Equipment. In the examination of the milk equipment on 60 cream producing farms it was found that 80.6 per cent (29 out of 36) of the milk separators, 76.7 per cent (46 out of 60) of the hand milk pails and 65.2 per cent (15 out of 23) of the strainers harbored *O. lactis*. The teat cups and milk tubes of 2 (5.1 per cent) out of 39 milking machines on farms that marketed fluid milk yielded *O. lactis*. A sample of turbid water containing milk solids secured from the vacuum line of a milking machine installation at a dairy barn gave a plate count of 145,000 *O. lactis* per ml. and a total mold and yeast count of 720,000 per ml.

Feed. The individual concentrates contained *O. lactis* in 32 (17.0 per cent) out of 188 samples while 50 concentrate mixtures prepared on farms harbored the mold in 25 (50.0 per cent) of the samples. Of the 141 samples of 5 different kinds of hay investigated 28 (19.9 per cent) yielded *O. lactis*; with lespedeza 15 (37.5 per cent) out of 40 samples contained the organism. This mold was found in 51 (66.2 per cent) out of 77 samples of silage but in only 2 (8.0 per cent) out of 25 samples of beet pulp. The lower, mature leaves of the corn plant in contact with the ground harbored *O. lactis* in 5 (16.7 per cent) out of 30 samples.

Miscellaneous Materials. Baled and loose straw contained *O. lactis* in 8 (20.0 per cent) out of 40 samples. Fresh cow feces, material from coat of cows, fresh horse feces, bedding and dirt from barn floors usually harbored *O. lactis* and ranked in that order in the per cent of positive samples yielded which ranged from 86.1 to 77.5 per cent respectively.

Soil. Of the 65 samples of barnyard soil investigated 51 (78.5 per cent) contained *O. lactis*. The organism was detected in 15 (28.8 per cent) of 52 samples of field soil and in 13 (46.4 per cent) of 28 samples of garden soil.

Water. *O. lactis* existed in 25 (29.8 per cent) of 84 samples of cistern water used for washing milk utensils on the farms where collected.

Plate Count of O. lactis in Fresh Cow Feces and Silage

Cow Feces. Samples of freshly excreted feces were collected in sterile containers from 60 individual cows in four herds during the summer months when consuming pasture grass and grain mixture and from the same number of cows in these herds during the winter months when they were fed hay, silage and grain mixture. The containers were not iced but the dilutions were made and the plates poured with tomato agar within 3 hours after the samples were collected.

TABLE 2.-SEASONAL VARIATION IN PLATE COUNT OF O. LACTIS IN FRESH COW FECES.

Season	Samples with plate count per gm. of: Less than 10		10 to 100		110 to 1000		1100 to 10,000		11,000 to 50,000		51,000 to 850,000		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Summer	25	41.7	7	11.7	8	13.3	18	30.0	2	3.3	0	0.0	60	100
Winter	0	0.0	1	1.7	7	11.7	11	18.3	21	35.0	20	33.3	60	100

The summary (Table 2) of the results shows that *O. lactis* is commonly present in fresh cow feces in numbers varying from a few hundred or less to several hundred thousand per gram. The numbers are much higher during the winter months when the cows consume hay and silage than during the summer when they are on pasture and receive no silage. During the summer months 25 (41.7 per cent) of 60 samples gave *O. lactis* counts of less than 10 per gm. while 18 (30.0 per cent) of the samples yielded counts between 1,100 and 10,000 per gm. The highest *O. lactis* count obtained from the summer samples was 12,000 per gm. Of the 60 samples plated during the winter months only 1 (1.7 per cent) had an *O. lactis* count of less than 100 per gm. while 21 (35.0 per cent) yielded counts between 11,000 and 50,000 per gm. and 20 (33.3 per cent) gave counts between 51,000 and 850,000 per gm. The samples from one herd gave consistently higher *O. lactis* counts on two examinations during winter feeding than samples from the other three herds. It was later learned that this herd was consuming silage with a very high *O. lactis* content (Table 2, sample 1).

When 10 samples of fresh cow feces were held at 25° C. for 24 hours and then replated it was found that the *O. lactis* counts had increased 8 to 12 times during holding, the final counts

ranging from 3.4 to 6.2 million per gram. Another group of 10 samples were placed in a water bath immediately after plating and heated to 38.5° C., the average rectal temperature of the cow, and held at that temperature for 48 hours then replated. No increase in the *O. lactis* count occurred in any of the samples.

Silage. One quart samples of silage were obtained in paper bags from silos during January and February when the surface layer of the silage was being removed daily for feeding. Water dilutions were prepared from 11 grams of the fresh silage and plated on tomato agar. The silage samples were then held 36 hours at 25° C. and replated in dilution up to 1:1,000,000.

TABLE 3.-PLATE COUNTS OF O. LACTIS IN FRESH AND AGED SILAGE

Sample No.	Type of silage	O. lactis plate count per gm. Fresh	Held 36 hrs. at 25° C.
1	corn	1,800,000	67,000,000
2	"	930,000	38,000,000
3	"	120,000	640,000
4	"	11,000	55,000
5	"	4,500	47,000
6	"	2,600	370,000
7	"	1,300	280,000
8	"	200	65,000
9	"	100	180,000
10	"	100	15,000
11	oat	1,200	240,000
12	sorgo	1,500	290,000
13	"	700	16,000
14	"	100	2,100
15	"	100	1,400
16	"	100	100
17	"	100	100
18	"	100	100
19	soybean	100	2,200
20	sudan grass	800	170,000

Since no data on the *O. lactis* plate count of silage has been encountered in the literature the results obtained for 20 samples of silage are recorded in Table 3. Some samples of fresh silage showed surprisingly high counts and as a rule the counts increased considerably during aging. In general corn silage usually gave higher plate counts in both the fresh and aged material than sorgo or other types of silage. One sample of corn silage and 4 samples of sorgo silage contained no *O. lactis* colonies on the plates poured from the 1:100 or higher dilutions of the fresh materials. Three of the aged sorgo samples gave negative plate counts for this organism. Some error in sampling was no doubt involved in the analysis of this material. The highest count on the 9 samples of fresh corn silage was 1,800,000 per

gram, while the maximum count on the 7 samples of fresh sorgo silage was 1,500 per gram. After aging, the counts on these two samples were 67,000,000 and 290,000 per gram respectively. Samples 2, 10 and 14 showed some evidence of spoilage but the other samples were normal in all respects.

Discussion of Results

The results denote that the concentration of *O. lactis* in the barn air depends on the kind and amount of dust in the air. When the air is relatively free of dust comparatively few spores, oidia or hyphae fractions of *O. lactis* are falling from the air and their numbers increase only slightly when a heavy dust concentration is raised by feeding hay or bedding down the stall or stanchion floor. This mold is, however, usually fairly abundant in the barn air near the cows when the animals are being brushed and cleaned, particularly when their coats are soiled with dust or dried excreta from the barn floor or yards. Milk or milk equipment exposed to the barn air at this time or before most of the dust raised by grooming the cows has settled from the air, could easily become contaminated with *O. lactis.*

The milk pails, strainers and milk separators used on farms that produce cream in commercial quantities commonly harbor *O. lactis* during the summer months. This is probably due to the fact that adequate facilities for washing, sterilizing and storing the milk equipment were not available on most farms. The milk utensils were usually washed in the kitchen and stored there or on the porch or outside benches exposed to dust and fly contamination. Since cistern water often contains *O. lactis* it may be a source of contamination of milk equipment washed or rinsed in this water unless the equipment is subsequently sterilized. The rubber milk tubes and teat cups of milking machines given the usual cleaning and sterilizing treatments practiced on dairy farms seldom contain *O. lactis.* However, improperly cleaned and sterilized rubber units and condensation water from the vacuum line may be a source of contamination with this organism.

While the common feeds stored on dairy farms frequently contain *O. lactis* they are usually not a relatively rich source of the organism. The number of reproductive elements of this mold on the different feeds probably depends on the extent of soil or dust contamination during growth or harvesting and on the amount of contamination in various ways during storage.

Silage of all kinds generally contains *O. lactis* and sometimes in rather large numbers, particularly corn silage. This mold evidently exists in small numbers on green plants at the time of ensiling and no doubt grows to some extent during early fermentation before the oxygen is exhausted and again when the silage is exposed to the air during feeding.

Both cow and horse feces generally harbor *O. lactis* and it is often present in the former in very large numbers, especially during the winter months when silage is included in the ration. The concentration of the organism in the feeds consumed evidently determines the number of reproductive elements of this mold in the freshly excreted material. The organism apparently does not grow in the rumen or other parts of the intestinal tract of the cow because of the high temperature. Considerable growth of the mold occurs in manure when the temperature is favorable and since it survives drying the dry or partially dried material is generally seeded with *O. lactis*. Through manure the organism becomes distributed to various places about the barn, especially to barn floors and bedding, barnyard soil and the coat of the cows. The chance addition of particles of foreign material of this type to milk would usually result in contamination with *O. lactis*. Clean production methods are, therefore, essential if the entrance of this mold into milk is to be prevented.

SUMMARY

The dust raised in the barn air by grooming the cows usually contains a significant number of spores, oidia or hyphae fractions of *O. lactis*. Hay and bedding dust in the air carries only a relatively small number of the reproductive elements of this mold. Comparatively clean barn air is relatively free of this organism.

On cream producing farms the milk equipment commonly harbors *O. lactis* during the summer months. It was isolated from 80.6 per cent of 36 separators, 76.7 per cent of 60 milk pails and 65.2 per cent of 23 strainers. On farms that marketed fluid milk the milk tubes and teat cups of 5.1 per cent of 39 milking machines yielded the organism.

The dry feeds stored on dairy farms frequently contain *O. lactis* in 10 grams of material. The mold was detected in 50 per cent of 50 concentrate mixtures and in 17.0 per cent of 188 individual concentrates. Of 141 samples of hay examined 19.9 per cent harbored the organism.

O. lactis usually exists in silage and often in large numbers, particularly in corn silage, due to growth under suitable conditions.

Cow manure is the major source of *O. lactis* on dairy farms. The organism is distributed by manure to various places about the barn and surroundings, especially to bedding, barn floor and barn yards and the coat of the cow. Material from the body of the cow falling into milk during the milking process probably comprises the most important source of *O. lactis* contamination of milk. Clean production methods, particularly clean cows, and sterile utensils should largely prevent the contamination of milk with this mold.

The organism was detected in 28.8 per cent of 52 samples of field soil and in 46.4 per cent of 28 samples of garden soil.

Cistern water contained *O. lactis* in 29.8 per cent of 84 samples investigated.

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